Transformation (from Jesse Zalatan, Lim Lab)

- References
  - Gietz & Schiestl, *Nature Protocols*, **2**, 31-34, 2007.
  - Gietz & Schiestl, *Nature Protocols*, **2**, 35-37, 2007.
- This protocol takes ~1 hr after harvesting cells.

Reagents – all aqueous solutions should be filtered

- YPD
- 1 M LiOAc (typically ~pH 8)
- 10X TE pH 7.5 (for 10X: 100 mM Tris pH 7.5, 10 mM EDTA)
- 1X TE pH 7.5, 0.1 M LiOAc (make from 10X stocks)
- 50% PEG 3350
- DMSO
- salmon sperm DNA (ssDNA)

Day before: grow yeast strain to be transformed in 5-10 mL YPD overnight at 30 °C.

Procedure

1. If transforming DNA for integration, set up restriction digest to linearize DNA. Use ~1 μg plasmid DNA, 37 °C 2 hours, place tubes on ice (no need to clean up digest).
   - Reaction time can be shortened to <30 min if necessary.
   - Typically use PmeI to linearize (see notes on integrating vectors below)
2. Dilute O/N culture ~1:20 in YPD. Grow 2-4 hours at 30 °C to OD_{600} = 0.4-0.6 (doubling time ~90 min, dilute culture starts ~OD 0.05-0.10).
   - Inoculate a total volume of YPD to provide final 2.5 mL yeast per rxn.
   - For sigma strains, aim for 0.5-0.6 for best yield. Too many cells dramatically reduces transformation efficiency (this does not seem to be true for W303 – works fine with OD 0.8-1.0).
3. Prepare ssDNA: boil for 10 min (sandblock), cool on ice for at least 10 min (10 μL of 10 mg/mL stock per transformation)
   - Avoid repeatedly boiling stock of ssDNA (boil aliquots, store at 4 °C)
   - 10 μL of 2 mg/ml ssDNA is sufficient for most transformations, but use 10 μL 10 mg/mL if yield is low (i.e. sigma strains with Leu selection).
4. Harvest cells in clinical centrifuge (using 15 mL conical tubes), 3000 rpm, 2-5 min
5. Wash (resuspend) with 1mL and transfer to a 1.5mL tube (culture volume) 0.1 M LiOAc in TE, pH 7.5 or water.
6. Pellet cells (3000 rpm, 2-5 min)
7. Resuspend pellet in 100 μL (0.1 M LiOAc in TE, pH 7.5) per 2.5 ml culture, split into 100 μL per microfuge tube for desired number of transformations.
8. To 100 μL cells, add 100 μg ssDNA (10 μL of 10 mg/mL stock), 1-5 μL of target DNA (~1 μg for integrating plasmids).
   - Less target DNA needed for CEN/ARS plasmids (use ~100 ng)
   - For deletions, use 15 μL of PCR product at 600 ng/μL (15-20 μL at 300 ng/μL also typically works fine -> ~100 colonies)

9. Add in order: 480 μL 50% PEG 3350, 60 μL 10X TE, 60 μL 1M LiOAc (for final 40% PEG, 1X TE, 0.1 M LiOAc)
    - Add 75 μL DMSO (can significantly increase yield ~10X)
10. Vortex
11. Incubate at 42 °C for 30 min (shaking not necessary). Begin drying plates now.
12. Pellet (6000 rpm 2 min in microfuge), discard SN (remove PEG completely by pipetting), resuspend in 500 μL YPD (or selective media).
    - Alternatively: do 2X YPD wash to get rid of PEG
13. Pellet, discard SN, resuspend in residual ~50 μL YPD (or selective media)
14. Plate on selective media (i.e. SD –His)
15. Incubate for 1-3 days at 30 °C
    - W303/S288C typically grow in 2 days. Sigma strains usually take 3 days.
    - At room temp, W303 takes ~4 days.
16. For CEN/ARS transformations, use directly from transformation plate within 1 week (can sometimes store plates at 4 °C for a few months). For integrations, proceed as follows:
    - Streak 3 colonies per transformation on patch plate (pick single colony, make a 1 cm streak).
    - Grow for 1-2 days at 30 °C, then store at room temp or 4 °C. Can now always go back to the same colony (patch). For storage longer than 1 month, patch on new plates or make glycerol stocks.
    - Verify integration by PCR (see protocol below).
    - After streaking once on selective media, strains with chromosomal integrations can subsequently be maintained on YPD.

Steps that should be followed precisely to optimize efficiency (no shortcuts)

1. Step 3 (boil/ice for carrier ssDNA)
2. Fresh 50% PEG 3350 (old PEG is evaporated – higher conc, and breakdown products), stored at 4 °C protected from light. Use within 3 months.
3. Remove PEG completely before resuspending in YPD

Transforming integrating plasmids (pNH600 series single integration vectors)
Constructed by Noah Helman (Lim lab) for reliable single integrations into yeast genome. pSV606 was constructed by Simon Vidal in the El-Samad lab after original pNH606 failed to work correctly. Jessica Walter constructed additional vectors with antibiotic resistance markers (JW607-9).

Available vectors:

- pNH603: integrates into HIS3 locus, using Candida glabrata HIS3 marker
- pNH604: integrates into TRP1 locus, using Candida glabrata TRP1 marker
- pNH605: integrates into LEU2 locus, using Candida glabrata LEU2 marker
- pSV606: integrates into URA3 locus, using Candida albicans URA3 marker
- pJW607: integrates into HO locus, HygB resistance
- pJW608: integrates into mfa2 locus, NatR resistance
- pJW609: integrates into mfa2 locus, KanR resistance

Features:

- Integrates only once into the genome. Multiple recombination events simply replace the original insert.
    - For comparison: the pRS series vectors can integrate multiple times because they are linearized by a single cut within the ORF of the complementation gene. Recombination produces one repaired marker and one defective marker (note this strategy only works in strains with point mutants as auxotrophic markers, not $\Delta 0$ complete ORF deletions). Multiple integrations produce multiple markers.
    - When using antibiotic resistance markers in strains that already have one antibiotic resistance cassette integrated, maintain selection for original cassette. The antibiotic markers share the same TEF promoter terminator cassette and can swap themselves out.
- Compatible with pRS multiple-cloning site (KpnI-PspOMI-XhoI-BamH1-EcoR1-NotI-SacI…)
- Compatible with AarI cloning (contains AD acceptor sites between Xho and Not)
- Compatible with yeast homologous recombination cloning
- Can be easily shuttled between CEN/ARS vector and integration vector using single 8-base cutter (AscI).
- Can all be digested for integration using the same restriction sites
    - Any one of the 8-base cutters FseI, PmeI, SwaI. Choose an enzyme that does not cut your insert. Typically use PmeI.
        - 606-609: only PmeI available
    - These enzymes cut twice. For example, in pNH603, the enzyme will cut outside of His3 5' UTR and outside of 3' UTR. Promoter, insert, and terminator are cloned in after His3 terminator, before His3 3' UTR. Single stranded DNA then recombines with defective His3 gene (pop in/pop out style). Any multiple recombination events simply replace original recombination insert.
    - Use restriction digest directly in yeast transformation reaction (~1 μg). No cleanup necessary.

- Uses markers from *C. glabrata* to avoid problems with homologous recombination "healing" the endogenous gene without incorporating entire vector.
- Built-in terminator taken from ADH1 gene from *C. albicans*.
- Standard AmpR backbone derived from pRS306, modified to remove unknown DNA that contained an extra AarI site.

Notes:

- These vectors have been designed to work with W303 strains, where the auxotrophic markers are typically point mutations. For strains derived from S288C and ∑1278b, where some of the auxotrophic markers are complete ORF + flanking region deletions, this integration strategy may fail.
    - pNH603 has been successfully used with *his3Δ0::hisG*
    - pNH605 has been successfully used with *leu2Δ0*
    - pSV606 has been successfully used with *ura3Δ0*
- pNH604 (Trp) uses homology within the TRP1 ORF and will not work with strains with *trp1Δ0* (ORF deletion), such as many ∑1278b strains.
- In W303, the Trp1 mutation is a single mutation and reversions are possible. False positive background colonies will be more frequent with Trp selection and it is particularly important to confirm integration by PCR with pNH604 constructs.
- Point mutant Trp and Ura alleles in W303 have the potential to revert - avoid putting potentially toxic constructs at these loci – you are more likely to get reversions than your insertion of interest.
- For antibiotic resistance (HygB/Kan/Nat): plate on YPD overnight, first thing next morning replica plate onto antibiotic selection.
    - Restreak for singles before patching
    - Alternatively: can outgrow in YPD for 2-4 hours, then plate on selective media.
    - If target strain already has another antibiotic resistance marker, maintain double selection.
- 607 integrates at HO (and part of SSB1) – removes capability for mating type switching
- 608/609 integrate at mfa2. Removal of mfa2 gene prevents MATa-type cells from making a-factor, may disrupt mating.